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Award Number: DAMD17-01-1-0318

TITLE: Immunotherapeutic Strategies in Breast Cancer: Preclinical and Clinical Trials

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REPORT DATE: September 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-09-2011	Annual	15 Aug 2010 -14 Aug 2011

4. TITLE AND SUBTITLE
Immunotherapeutic Strategies in Breast Cancer: Preclinical and Clinical Trials

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-01-1-0318

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mayo Clinic Foundation
Scottsdale, AZ 85259

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project is focused on novel tumor vaccines directed at MUC1 and other tumor antigens. Our specific aims are: 1) To assess the effectiveness of vaccines against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice; 2) To translate an effective vaccine strategy into a phase I clinical trial in patients with undetectable disease following standard therapy. The model of spontaneous mammary cancer is the MUC1-expressing polyoma middle T antigen mice (MMT). We have tested five vaccines in the preclinical mouse model and all elicited a strong immune response. The vaccine using MUC1 class I binding peptides prevented MUC1-expressing tumor growth. We have designed the Phase I clinical trial using a peptide vaccine comprised of MUC1 and HER-2/neu MHC class I peptides and HER-2/neu MHC class II peptide with unmethylated CpG oligodeoxynucleotides and GM-CSF as adjuvants in breast cancer patients free of disease. The clinical trial was unanimously approved by the Mayo Institutional Review Board (IRB 582-05) following receipt of FDA approval (BB-IND 12155) and by the DoD HSRRB in January 2007 and HRPO on June 9, 2008. The clinical trial opened August 28, 2008. Thirty-nine patients have been enrolled. The trial is closed to accrual.

15. SUBJECT TERMS
Immunotherapy, vaccine, MUC1, mucin, mouse model, tolerance

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	13	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

This project is focused on the development of novel tumor vaccines directed at MUC1, a transmembrane mucin that is aberrantly expressed in cancer. MUC1 is expressed on greater than 90% of breast cancers and often elicits cellular and humoral immune responses in humans. However, these responses are not sufficiently strong to eradicate tumors. MUC1 is a candidate peptide for novel immunotherapy strategies to strongly activate the immune system to eradicate tumors expressing these epitopes. In tumors, there is strong over expression of MUC1 on tumor cells and in circulation, expression is no longer restricted to the apical domain of cells, and glycosylation is altered, revealing immunodominant tumor-specific peptide sequences.

In our preclinical studies we have utilized mice that develop spontaneous mammary gland cancer that express MUC1. MUC1 transgenic mice (MUC1.Tg) were bred with mice carrying the MMTV-driven polyoma middle T antigen (MT) to create MMT mice. Mice transgenic for this protein develop B and T cell tolerance and are refractory to immunization with the protein encoded by the transgene. All mice are congenic on the C57BL/6 background to eliminate strain-specific modifier effects. In the MMT mice, mammary gland tumors are induced by the action of a potent tyrosine kinase activity associated with the polyoma virus middle T antigen driven by the mouse mammary tumor virus long terminal repeat (MMTV) [2]. Middle T specifically associates with and activates the tyrosine kinase activity of a number of c-src family members, eliciting tumors when a threshold level of gene product has been attained. This promoter is transcriptionally active throughout all stages of mammary gland development and results in widespread transformation of the mammary epithelium and the rapid production of multifocal mammary adenocarcinomas in 100% of the female mice. The MMT mouse appears to be an appropriate model for human cancer and allows us to study the effects of self-tolerance, immunity and auto-immunity to MUC1 as mammary tumors develop spontaneously.

The **hypothesis** of our study is that enhancing MUC1-specific immunity will result in anti-tumor immunity. We propose to develop an optimal cancer vaccine using epithelial cell mucin MUC1 peptides or protein or MUC1-expressing tumors presented by DCs as immunogen. The most successful therapies will be tested in a phase I clinical trial. An additional hypothesis is that tolerance occurs within the tumor environment, although immunization strategies can be developed to overcome tolerance.

RESULTS (BODY)

Specific Aims:

Specific Aim 1: To assess the effectiveness of vaccine formulations against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice.

Our preclinical studies were completed at the end of year three and the papers describing the results were included in the progress report for 2006.

Specific Aim 2: To translate the most effective vaccine strategies into phase I clinical trials in patients with high and low tumor burden.

Section II - Description of Regulatory Status of the Clinical Trial

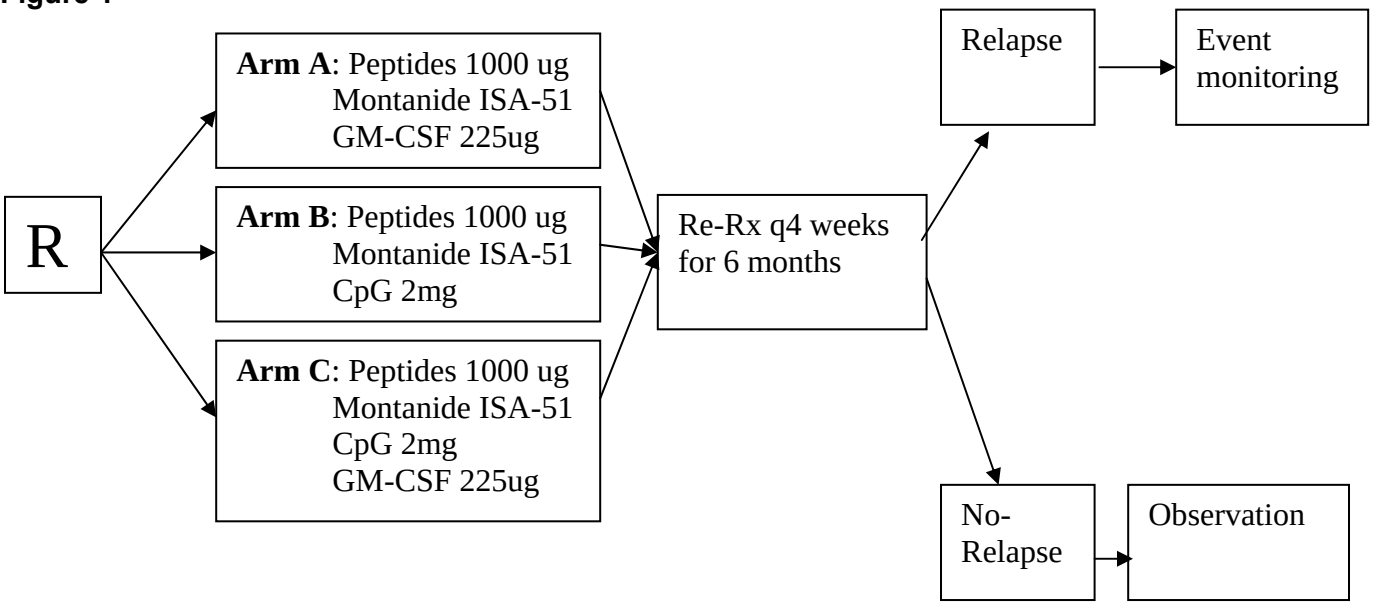
The clinical trial opened August 28, 2008, at all three Mayo Clinic sites. On September 13, 2010, 39 patients have been enrolled. Arm A closed June 30, 2010 as accrual was met. Arm B was temporarily closed on November 4, 2009 and was permanently closed on June 30th, 2010, due to dose limiting toxicity (local injection site reaction) of regimen. Arm C was temporarily closed on February 23, 2009 and then permanently closed June 30th, 2010 for dose limiting toxicity (local injection site reaction) of the regimen. All patients have completed injections and are being followed-up.

The trial is testing the MUC1 class I peptide (STAPPVHNV), HER-2/neu class I peptide (ILHNGAYSL) and HER-2/neu class II peptide (KVPIKWMALESIL) (1000 µg of each peptide) delivered in Montanide ISA-51 and comparing GM-CSF with unmethylated CpG oligodeoxynucleotides (PF 3512676) as immune adjuvants. Few vaccines have been tested in the optimal setting of minimal residual disease. CpG

unmethylated oligodeoxynucleotides are a novel adjuvant that promote strong, antigen-specific T cell responses and help to overcome immune tolerance.

The schema for the clinical trial is shown (Fig. 1).

Figure 1



Recruitment Plan and No-Cost Extension

The protocol MCO338 entitled "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas" opened on August 28, 2008. The trial was closed briefly from 2/24/09 until 5/19/09 due to toxicity. The dose of CpG ODN (PFT PF 3512676) was reduced to 1 mg (from 2 mg) in arms B and C and there have been no additional reports of toxicity. In that time we have accrued 39 patients by June 30, 2010, when the trial was permanently closed to enrollment. Presently, no patients are receiving injections and patients are being followed-up. Since the described trial includes a two-year follow-up period, with blood draws and immune monitoring occurring until 2 years after the first vaccination, we are on schedule to complete our studies by August 14, 2012 (the date of our no-cost extension).

Eligibility and Enrollment

This study enrolled women 18 years of age or older with histologically confirmed invasive breast cancer surgically treated within 3 years of enrollment who completed adjuvant treatment with chemotherapy and /or radiation therapy and who had no radiographic evidence of disease. Additional eligibility criteria included: MUC1 positive breast cancer by central pathology evaluation, HLA-2A positive serotype, ECOG PS of 0-1, and adequate hematologic, renal and hepatic function. Exclusion criteria included: active infection; known to be HIV positive, have hepatitis, or an immunocompromising condition; concurrent anti-cancer standard or investigational therapies; or another invasive malignancy within 5 years of enrollment. Women who were pregnant or breast feeding were not eligible for participation.

Study treatment

All patients were vaccinated subcutaneously in a non-dissected lymph node region with a mixture of 1 mg of MUC1 class I peptide (STAPPVHNV); 1 mg of HER-2 class I peptide (ILHNGAYSL) and 1 mg HER-2 peptide class II peptide (KVPIKWMALESILRRRF) suspended in 1.5 mL of Montanide ISA-51. Forty-five patients (15 patients per immunization strategy) were to be randomized to receive the vaccine with 0.225 mg of GM-CSF (Arm A) or the vaccine with 2 mg PF-3512676 (previously known as CpG) (Arm B) or vaccine with both 0.225

mg of GM-CSF and 2 mg PF-3512676 (Arm C) day 1 of a 28 day cycle for a maximum of 6 cycles. The dose of PF-3512676 (CpG) was lowered to 1 mg with implementation of Addendum 1.

At any time prior to registration, patients submitted a tumor specimen for central laboratory testing of MUC1 and HER-2 expression and blood for HLA serotyping. Only patients found to have MUC1 positive disease by central testing were allowed to register on this trial. Within 14 days of study registration, prior to each treatment cycle, 4 weeks after completion of treatment, and every 3 months thereafter until progression or a maximum of 2 years post registration, patients underwent a complete physical exam, assessment of performance status, blood chemistries, toxicity assessments (Common Terminology Criteria for Adverse, Events, CTCAE v. 3.0), and research blood draws for immunologic profiling. Disease status was radiographically evaluated at registration and per standard of care by treating physician. DTH skin testing for mumps, candida, tetanus toxoid, and trichophyton was done prior to treatment and at treatment cycle 6.

Treatment was discontinued if a patient developed a grade 2+ allergic reaction, autoimmune reaction; or neurologic difficulties or any grade 3+ adverse event. Treatment discontinuation for a grade 2 injection site reaction was added as part of Addendum 1. All patients received standard supportive care, including antibiotics, transfusions, and treatment of other newly diagnosed or concurrent medical conditions.

Statistical considerations

The study was designed to examine the safety profile and immunization efficacy of each immunization strategy. Fifteen patients were randomized to each strategy using a dynamic allocation procedure that balanced the marginal distributions of dominant disease between treatment arms. The number and severity of all toxicities reported to be possibly, probably, or definitely related to treatment were reported using the NCI-CTC version 3.0 criteria. The immunologic parameters of interest were the change in the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and dendritic cells and the number of peptide specific IFN-gamma producing T cells and peptide-specific IL-5 producing T cells.

RESULTS

Study Course

Between October 1, 2008, and July 1, 2010, 35 women were entered onto this trial. One patient was found to be ineligible having had an ocular melanoma within 5 years of registration. After the first 13 patients (Arm A-4 pts; Arm B-4 pts, and Arm C- 5 pts) had been enrolled, it was noted that grade 2 injection site reactions (ISR) had occurred in 1 Arm A patient on cycle 1; 2 Arm B patients on cycle 3; 2 Arm C patients on cycle 1; and 1 Arm C patient on cycle 3. The trial was amended on May 19, 2009 to lower the dose of PF-3512676 to 1 mg for Arm B and Arm C. Patients enrolled after May 19, 2009 will be referred to as the postAdd1 patients. Enrollment to Arm B and Arm C closed early as the pre-specified toxicity safely boundary was crossed. Enrollment to Arm B was closed on November 9, 2009, as one of the 5 postAdd1 patients developed a grade 3 ISR and enrollment to Arm C was closed on February 3, 2010 as two of the 7 postAdd1 patients developed a grade 2 ISR. Enrollment to Arm A closed on July 1, 2010 having met its accrual goal.

The patient and tumor characteristics of the 34 eligible patients enrolled are presented in Table 1 by treatment arm.

Table 1. Patient and Tumor Characteristics

	Arm A n=16	Arm B n=11	Arm 6 n=11
median age (range)	52 (35-75)	58 (42-86)	54 (32-69)
group pre-Addendum 1 post Addendum 1	 10 (62.5%) 6 (37.5%)	 5 (45.5%) 6 (54.6%)	 7 (63.6%) 4 (36.4%)
estrogen receptor positive negative	 11(68.5%) 5 (38.5%)	 8 (72.7%) 3 (27.3%)	 7 (63.6%) 4 (36.4%)
Her2 expression positive negative	 4 (25.0%) 12 (75.0%)	 3 (27.3%) 8 (72.7%)	 3 (27.3%) 8 (72.7%)
Extent of surgery mastectomy lumpectomy ------	 3 (18.8%) 12 (75.0%) query - 1	 2 (18.2%) 4 (36.4%) query - 5	 3 (27.3%) 8 (72.7%)
Adjuvant therapy chemotherapy radiation therapy	 15 (93.8%) 12 (75.0%)	 10 (90.9%) 5 (45.5%)	 11 (100%) 10 (90.9%)
DTH testing candida positive tetanus positive	 1 (6.3%) 9 (56.3%)	 3 (27.3%) 7 (63.6%)	 4 (36.4%) 8 (72.7%)
Concurrent mediations NSAIDS ACE inhibitors Steroids Thyroid hormones beta blockers	 8 (50%) 1 (6.3%) 1 (6.3%) 3 (18.8%) 3 (18.8%)	 3 (27.3%) 2 (18.2%) 1 (9.1%) 2 (18.2%) 4 (36.4%)	 4 (36.4%) 0 0 3 (27.3%) 0
Signs/Symptoms grade 2 fatigue grade 2 rash grade 2 joint pain	 1 (6.3%) 1 (6.3%) 0	 1 (9.1%) 0 1 (9.1%)	 0 0 0

Treatment Course and Toxicities

Arm A

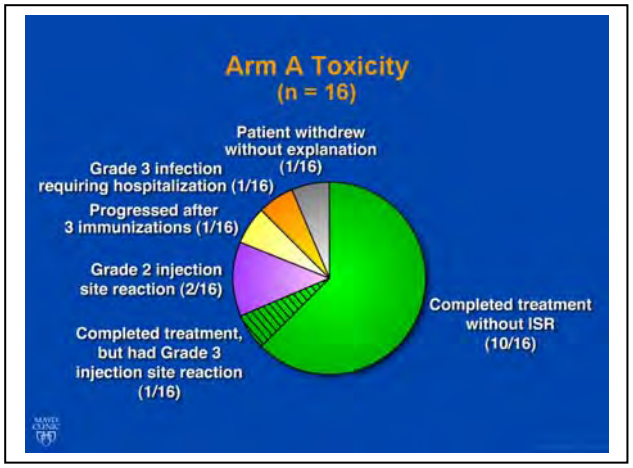
Eleven of the 16 patients randomized to Arm A completed all 6 immunizations. Three patients discontinued treatment due to adverse events, namely, grade 2 ISR (2 post Add 1 pts.) and a grade 3 infection (bladder) requiring hospitalization (1 pre Add pt.); 1 patient refused to have her last immunization; and 1 patient progressed after 3 immunizations. One other instance of a moderate to severe toxicity (grade ≥ 2) was reported. It was a preAdd1 patient who developed a grade 2 ISR with her first immunization and completed her remaining 5 immunizations without incident.

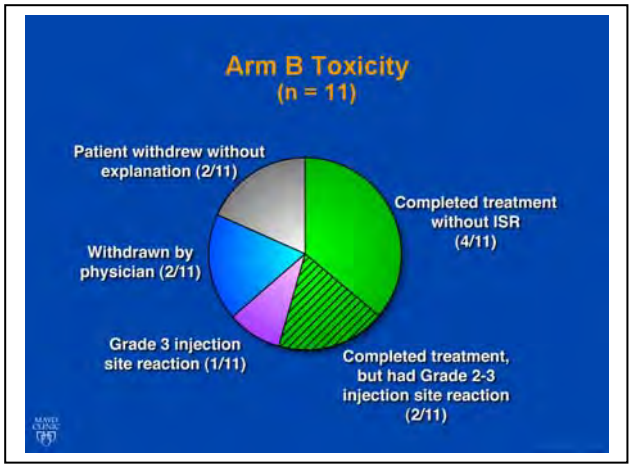
Arm B

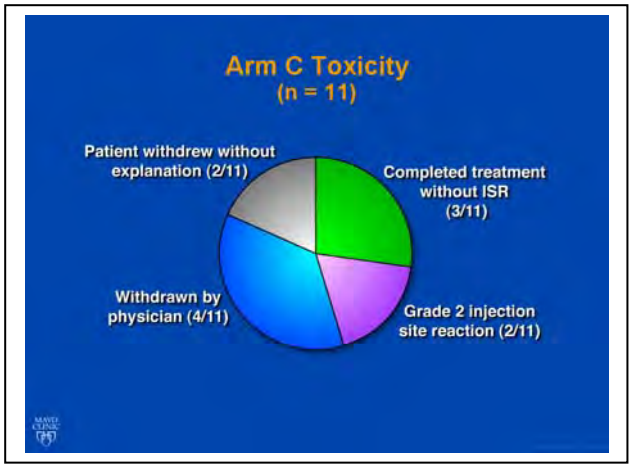
Eleven patients (6 preAdd 1 pts) were randomized to Arm B. Of the 6 preAdd 1 patients, 3 completed all 6 immunizations, 1 refused to continue participation; and 2 discontinued after their first immunization on the recommendation of the study team as safety issues with PF-3512676 were emerging. Of the 5 postAdd 1 patients, 3 completed all 6 immunizations, 1 refused to continue participation; and 1 discontinued due to grade 3 injection site reaction. Two other moderate to severe toxicities were reported. Both were a grade 2 ISR reported by preAdd1 patients who went on to complete all their remaining immunizations.

Arm C

Eleven patients (4 preAdd1) were randomized to Arm C. All 4 preAdd1 patients discontinued after 2, 3, 3, and 5 immunizations, respectively, on the recommendation of the study team as safety issues with PF-3512676 were emerging. Among the 7 post Add1 patients, 3 completed all 6 immunizations, 2 patients discontinued treatment both due to grade 2 ISR; and 2 patients refused to continue participation both after 3 immunizations. Other moderate to severe toxicities reported included: grade 2 ISR (3 preAdd pts.), grade 2 bone pain (1 preAdd 1 pt.); grade 2 fatigue (1 postAdd 1 pt.); grade 2 arthralgia (1 postAdd 1 pt.); and grade 2 myalgia (2 postAdd 1 pts.).







Clinical Outcome

Among the 38 eligible patients enrolled on this trial, one (Arm A) patient has progressed and died. The remaining 37 patients are alive without disease progression with a median follow-up time of 1.7 years (range: 2 month to 2.5 years).

Immunologic changes in response to MUC1 and HER-2 peptides

Twenty-nine of the 38 patients had blood draws taken prior to immunization and at least one time after randomization (either 2, 4, 6, 9 and/or 12 months on study). Peripheral blood mononuclear cells were isolated from the blood and were used to determine both the percentage of $CD8^+$ T cells binding the MUC1 tetramer and the percentage of $CD8^+$ T cells binding the HER-2 tetramer (Table 2).

A 2-fold or more increase in the percentage of MUC1-specific $CD8^+$ T cells was seen among 3 of 12 Arm A pts; 7 of the 8 Arm B pts (3/5 preAdd1; 3/3 postAdd1); and 5 of the 9 Arm C pts (3/4 preAdd1; 2/5 postAdd1). A 2-fold or more increase in the percentage of Her2 specific CD8+ T cells was seen among 5 of 12 Arm A pts; 6 of the 8 Arm B pts (4/5 preAdd1; 2/3 postAdd1); and 5 of the 9 Arm C pts (4/4 preAdd1; 1/5 postAdd1).

Among the 18 patients whose M1:M2 ratio was less than 1 prior to immunization, 8 patients (4/9 Arm A; 0/2 preAdd1Arm B; 3/3 postAdd1 Arm B; 0/2 preAdd1Arm C; 1/2 postAdd1 Arm C)
reported at least one post-randomization M1:M2 ratio > 1.5.

Among the 20 patients whose DC1:DC2 ratio was less than 1 prior to immunization, 4 patients
2/9 Arm A; 1/3 preAdd1Arm B; 0/1 postAdd1 Arm B; 1/3 preAdd1Arm C; 0/4 postAdd1 Arm C)
reported at least one post-randomization DC1:DC2 ratio > 1.5.

	Arm	N	(MUC1 response, HER-2 response)			
			(yes, yes)	(yes, no)	(no, yes)	(no,no)
	A	12	3	0	2	7
	B (preAdd 1)	5	4	0	0	1
	B (postAdd 1)	3	2	1	0	0
	C (preAdd 1)	4	3	0	1	0
	C (postAdd 1)	5	1	1	0	3

- MUC1 response defined as 2-fold or more increase in the percentage of MUC1 specific $CD8^+$ T cells from pre-immunization levels
- HER-2 response defined as 2-fold or more increase in the percentage of HER-2 specific $CD8^+$ T cells from pre-immunization levels

KEY RESEARCH ACCOMPLISHMENTS

- The preclinical research was completed and described in the annual reports for years 3 and 4.
- Among the 38 patients enrolled, one patient (Arm A) developed tumor progression and died. The remaining 37 patients are alive without disease progression with a median follow-up time of 2 years and 3 months (range 5 months to 2.8 years).
- Arm A met the accrual goal. Arms B and C closed early as the pre-specified toxicity safety boundary was crossed.
- There was significant adjuvant-dependent sensitization to MUC1; a similar trend was observed for HER-2/neu.
- Arm B (CpG) was significantly better than Arm A (GM-CSF) (p=0.02, Fisher's exact 2-tailed test)
- Combined arms with CpG (Arms B, C) were significantly better than no CpG (Arm A) (p=0.03).
- Arm B (CpG only) was significantly better than combined Arms with GM-CSF (Arms A, C) (p=0.04).
- For HER-2^+ tetramers, a similar trend was observed.
- There were no consistent changes observed in cytokine levels or percentages of $CD4^+$, $CD8^+$ or myeloid cell populations. Additional immunological studies are still in progress.

REPORTABLE OUTCOMES

- The Clinical Trial was activated on August 28, 2008, at Mayo Clinic Arizona, Mayo Clinic Rochester, Minnesota and Mayo Clinic Jacksonville, Florida.
- The clinical trial has enrolled 39 of the 45 anticipated patients (87%). The trial was permanently closed to enrollment on June 30, 2010.
- All patients have completed injections and are being followed-up.

Time Table of Protocol Development

- Clinical protocol concept approved by Mayo Clinic Cancer Center 12-11-03
- Completed Mayo Clinic Cancer Center Peer Review process 5-4-04
- List of recommendations by FDA (pre IND conference) 4-21-04
- Peptides synthesized and vialed by ClinAlfa® for use in this clinical trial:
  1. Her-2/neu (435-443)
  2. Her-2/neu (883-899)
  3. MUC1 (950-958)
- Completion of IND documentation and submission to FDA on December 17, 2004.
- FDA approval (IND # 12155)
- Mayo IRB approval April 22, 2005 (IRB 782-05)

- Submission to DOD HSRRB on May 11, 2005
- Submission to FDA of the revised 1572 and Investigator's Brochure on September 15, 2005
- Submission to Mayo IRB of amendment, which excludes prisoners from the study population and reduces the number of personnel involved in the study (September 12, 2005)
- Submission of revision to HSRRB on February 10, 2006 (response to request for revisions from 14 December 2005 HSRRB meeting)
- Submission of revision to HSRRB on May 18, 2006
- Submission of final documents to the Mayo IRB August 30, 2006
- Final approval Mayo IRB December 15, 2006
- Final approval DoD HSRRB (Log Number A-10856) January 26, 2007
- CpG-7909 adjuvant to be supplied by Pfizer as PF3512676 for this clinical trial.
- Submission of revised clinical protocol to Mayo IRB, HSRRB and IND (July, August 2007)
- Final Mayo IRB approval August 2, 2007
- Extension of "performance period" by 24 months to 14 September 2009
- Approval from DOD HRPO on June 9, 2008
- Clinical Trial was activated August 28, 2008.
- Clinical Trial was closed from 2/24/09 to 5/19/09 due to toxicitiy at injection sites. The amount of CpG ODN adjuvant was reduced from 2 mg to 1 mg (Addendeum 1) and the trial was reopened.
- Enrollment has been completed and the trial was closed June 30, 2010. Thirty-nine patients out of the anticipated 45 patients (87%) were enrolled. Two of the arms (B and C) were closed prematurely (June 30, 2010) due to dose limiting toxicities (injection site reaction toxicity > CTC grade 2).

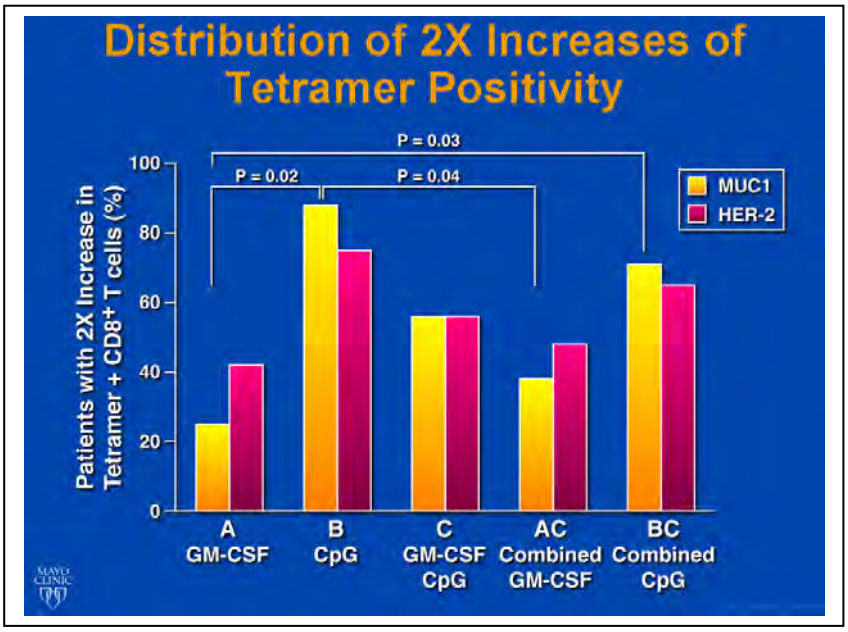
CONCLUSIONS

The trial opened August 28, 2008 and it has accrued 39 patients by June 30, 2010, when it was permanently closed to enrollment. Accrual was met for Arm A. Arms B and C were closed after accrual of 11 and 12 patients, respectively. Arm B was temporarily closed on November 4, 2009 and was permanently closed on June 30th, 2010 due to dose limiting toxicity of the regimen (injection site reactions toxicity > CTC grade 2). Arm C was temporarily closed on February 23, 2009 and then permanently closed June 30th, 2010 for intolerability of regimen. As of September 13, 2011, enrolled patients are under observation and follow-up. We are on course to complete follow-up and immunologic analyses by August 14, 2012, the end-date of this study.

Among the 38 eligible patients enrolled, one patient (Arm A) developed tumor progression and died. The remaining 37 patients are alive without disease progression with a median follow-up time of 2 years (range 5 months to 2.8 years). Arm A met the accual goal. Arms B and C closed early as the pre-specificied toxicity safety boundary was crossed. It is important to note that CTC criteria did not previously separate injection site reaction from allergic reaction. Under the new guidelines, we could have completed accrual. When a

relevant autoantigen is introduced, the CpG toxicity observed was greater than previously reported. Higher toxicity may be due to efficacy of the immune response. Feasibility studies need to be developed to achieve tolerability at the same time that effective immunization is achieved.

There was significant adjuvant-dependent sensitization to MUC1; a similar trend was observed for HER-2/neu (see figure below). Arm B (CpG) was significantly better than ArmA (GM-CSF) (p=0.02, Fisher's exact 2-tailed test). Combined Arms with CpG (Arms B,C) were significantly better than no CpG (Arm A) (p=0.03). Arm B (CpG only) was significantly better than combined Arms with GM-CSF (Arms A,C) (p=0.04). For HER-2^{+} tetramers, a similar trend was observed. GM-CSF did not appear to be as effective an adjuvant as CpG. In contrast to the results with tetramers, there were no consistent changes observed in cytokine levels or percentages of $CD4^{+}$, $CD8^{+}$ or myeloid cell populations. Additional immunological studies are still in progress.



APPENDIX

Era of Hope Poster presented 3rd August 2011 in Orlando, Florida and a platform talk was given on 5th August 2011.

MUC1/HER-2/neu Peptide-Based Immunotherapeutic Vaccine Clinical Trial for Breast Adenocarcinoma

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Abstract

MUC1 is an excellent target for immunotherapeutic strategies for breast cancers. MUC1 was recently selected as the number 2 cancer vaccine target antigen, based in part on its immunogenicity, oncogenicity, and wide-spread cell surface expression. More than 90% of breast cancers over express aberrantly glycosylated MUC1 with altered cellular distribution. Although MUC1 stimulates both humoral and cellular immunity, these natural immune responses are often not efficient in eradicating tumors. We have designed a peptide-based cancer vaccine, utilizing MUC1 (class I) and HER-2/neu (class I and II) peptides in conjunction with strong adjuvants to enhance the immune response. A phase I/II clinical trial has completed enrollment of 39 patients with resected breast cancers who have completed standard adjuvant therapy. Patients (HLA-A*0201 with MUC1-expressing tumors) were randomized to one of three treatment arms: 3-peptide vaccine emulsified in Montanide ISA-51 with (1) GM-CSF, (2) CpG-7909 (PF-3512676), or (3) GM-CSF and CpG-7909. Patients were vaccinated subcutaneously every four weeks for six months and bloods collected every 6 months for the next 18 months. Patients are presently in the follow-up period. Vaccine immune responses will be monitored by staining peripheral blood samples with MHC tetramers containing the peptides of interest. Changes in multiple plasma cytokines and peripheral blood immune cell subsets are monitored by multiplex quantification and flow cytometry, respectively. Development of an antibody response and epitope spreading will be determined. Preliminary results show strong stimulation of MUC1 and HER-2/neu tetramer positive T cells by the vaccine, with up to 1% of CD8+ T cells that are MUC1- and HER-2/neu tetramer positive. Patients are being monitored prospectively for survival and recurrence.

Background

- **MUC1 peptide:** MUC1 was recently ranked 2nd out of 75 tumor-associated antigens by NCI as a promising target for cancer vaccine development. MUC1 is a cell-associated mucin expressed on the cell surface of many epithelial and hematological malignancies. Greater than 90% of breast carcinomas express MUC1; high levels are also found in adenocarcinomas originating from most tissues. Compared with MUC1 expressed by normal epithelial cells, tumor-associated MUC1 exhibits abnormal cell surface expression and glycosylation. Cancer patients have exhibited spontaneous antibody and T cell immune responses to MUC1; these are detected in about 10% of individuals. We hypothesize that vaccination using an HLA-A2-binding peptide from the tandem repeat sequence of MUC1 (**STAPPVHNV**) will elicit potent cytotoxic T lymphocyte (CTL) immune responses capable of recognizing MUC1-bearing tumor cells.
- **Her-2/neu peptides:** HER-2/neu is overexpressed in approximately 30% of breast cancer patients. Humoral and cellular immune responses to HER-2/neu have been detected in a minority of patients with advanced stage breast and ovarian cancer; several HLA-class I binding peptides have been identified, including the peptide used in this study (ILHNGAYSL). In addition to class I epitopes, immunogenic HLA-DR restricted class II epitopes have been defined for HER-2/neu. A promiscuous MHC class II TH epitope (**KVPIKWMALESILRRRF**) has been identified that elicits T cell responses restricted by HLA-DR1, HLA-DR4, HLA-DR52, and HLA-DR53. Peptide-induced T cells were effective in recognizing naturally processed HER-2/neu protein. HER-2 peptides were designed by Dr. Esteban Celis (*Human Immunol.* 1998; *Cancer Res.* 2000).
- **GM-CSF:** GM-CSF is a key mediator of dendritic cell (DC) maturation and function; it increases surface expression of class I and II MHC molecules as well as co-stimulatory molecules of dendritic cells *in vitro*. Addition of GM-CSF to peptide antigens may substantially enhance their immunogenicity. In a preclinical model, we have demonstrated that GM-CSF added to Montanide ISA 51 increases the frequency of interferon gamma (IFNg) secreting antigen-specific CTL in immunized mice, making GM-CSF an attractive immune adjuvant.
- **PF3512676 (CpG-7909):** Bacterial DNA possesses unique immuno-modulatory features of potential utility in cancer therapy. Synthetic oligodeoxynucleotide (ODN) constructs containing unmethylated CpG motifs are able to activate DC through ligation of TLR-9, enhancing their antigen processing/presentation properties and stimulating production of T helper 1 (Th1) cytokines needed for effective CTL immune responses. Several preclinical and clinical studies illustrate the ability of CpG ODN to function as potent vaccine adjuvants.

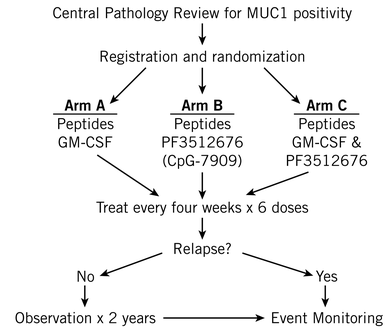
Study Goals

- To examine the safety profile and immunization efficacy of three vaccine strategies: Vaccine with GM-CSF, vaccine with CpG, or vaccine with GM-CSF and CpG.

Methods

- **Patient Population:** Patients with MUC1+ resected stage I (triple negative), II or III breast cancer who had completed all standard adjuvant chemotherapy and radiation therapy, ECOG 0-2, HLA-A2+, and ≥ 18 years old were included. Pregnant or nursing patients and patients with severe infection, recent therapy, or marked blood test abnormalities were excluded.
- **Treatment Administration and Evaluation:** Upon registration, patients were randomized to receive vaccine with GM-CSF (Arm A), CpG (Arm B), or GM-CSF and CpG (Arm C). Patients were treated every four weeks for six cycles or until disease progression.
- **Vaccine Preparation:** GM-CSF (225 µg) and CpG solutions (2 mg or 1 mg; 0.5-0.65 mL) were added to lyophilizecd peptide mixture (1 mg each peptide); then 1 mL Montanide ISA-51 was added, and the mixture was emulsified by vortexing for 12 minutes. The total vaccine volume was 1.5-2 mL.
- **Toxicity Assessment:** NCI Common Toxicity Criteria for Adverse Events v.3.
- **Immunologic Monitoring:** Serial peripheral blood plasma and PBMC samples were obtained. Immunophenotyping of PBMC and tetramer quantification of antigen-specific CTL were performed using standard flow cytometry techniques. Protein levels for 27 cytokines, chemokines, and growth factors were measured using the Bio-plex cytokine assay (Bio-Rad, Hercules, CA).

Schema



Patient and Tumor Characteristics

Characteristic	Arm A n = 16	Arm B n = 11	Arm C n = 11
Median age (Range)	52 (35-75)	58 (42-86)	54 (32-69)
Group			
Pre-Addendum 1	10 (62.5%)	5 (45.5%)	7 (63.6%)
Post-Addendum 1	6 (37.5%)	6 (54.6%)	4 (36.4%)
Estrogen receptor			
Positive	11(68.5%)	8 (72.7%)	7 (63.6%)
Negative	5 (38.5%)	3 (27.3%)	4 (36.4%)
Her2 expression			
Positive	4 (25.0%)	3 (27.3%)	3 (27.3%)
Negative	12 (75.0%)	8 (72.7%)	8 (72.7%)
Extent of surgery			
Mastectomy	3 (18.8%)	2 (18.2%)	3 (27.3%)
Lumpectomy	12 (75.0%)	4 (36.4%)	8 (72.7%)
Adjuvant therapy			
Chemotherapy	15 (93.8%)	10 (90.9%)	11 (100%)
Radiation therapy	12 (75.0%)	5 (45.5%)	10 (90.9%)
DTH testing			
Candida positive	1 (6.3%)	3 (27.3%)	4 (36.4%)
Tetanus positive	9 (56.3%)	7 (63.6%)	8 (72.7%)
Concurrent mediations			
NSAIDS	8 (50%)	3 (27.3%)	4 (36.4%)
ACE inhibitors	1 (6.3%)	2 (18.2%)	0
Steroids	1 (6.3%)	1 (9.1%)	0
Thyroid hormones	3 (18.8%)	2 (18.2%)	3 (27.3%)
Beta blockers	3 (18.8%)	4 (36.4%)	0
Signs/Symptoms			
Grade 2 fatigue	1 (6.3%)	1 (9.1%)	0
Grade 2 rash	1 (6.3%)	0	0
Grade 2 joint pain	0	1 (9.1%)	0

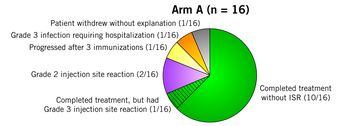
Results

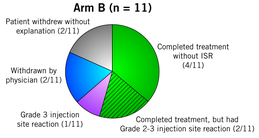
Immunzations resulted in increased numbers of CD8+ cells that bound MUC1 and HER-2 tetramers

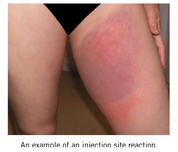
Arm of Study	n	(MUC1 Response, Her-2 Response) (Yes, Yes)	(Yes, No)	(No, Yes)	(No, No)
A	12	3	0	2	7
B (PF 2 mg)	5	4	0	0	1
B (PF 1 mg)	3	2	1	0	0
C (PF 2 mg)	4	3	0	1	0
C (PF 1 mg)	5	1	1	0	3

- MUC1 response defined as 2 fold or more increase in the percentage of MUC1 specific CD8+ T cells from pre-immunization levels
- Her2 response defined as 2 fold or more increase in the percentage of Her2 specific CD8+ T cells from pre-immunization levels

Toxicity Assessment



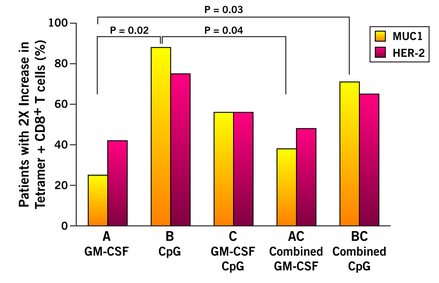




An example of an injection site reaction

Several issues prevented completion of the immunization protocol. In addition to injection site reactions (ISR), other toxicities reported in Arm C included grade 2 bone pain, grade 2 fatigue, grade 2 arthralgia, grade 2 myalgia.

Distribution of 2X Increases of Tetramer Positivity



Conclusions

- Among the 38 eligible patients enrolled, one patient (Arm A) has developed tumor progression and died. The remaining 37 patients are alive without disease progression with a median follow-up time of 1.7 years (range: 2 months to 2.5 years).
- Arm A met the accrual goal; Arms B and C closed early as the pre-specified toxicity safety boundary was crossed.
- There was significant adjuvant-dependent sensitization to MUC1; a similar trend was observed for HER-2.
- For MUC1+ tetramers:
  - Arm B (CpG) was significantly better than Arm A (GM-CSF) (P = 0.02, Fisher's exact 2-tailed test)
  - Combined Arms with CpG (Arms B, C) were significantly better than no CpG (Arm A) (P = 0.03)
  - Arm B (CpG only) was significantly better than combined arms with GM-CSF (Arms A,C) (P = 0.04)
- For HER-2+ tetramers, a similar trend was observed.
- When a relevant autoantigen is introduced, the CpG toxicity observed was greater than previously reported. Higher toxicity may be due to efficacy of the immune response. Feasibility studies need to be developed to achieve tolerability at the same time that effective immunization is achieved.
- GM-CSF did not appear to be as effective an adjuvant as CpG.
- In contrast to tetramers, there were no consistent changes observed in cytokine levels or percentages of CD4+, CD8+ or myeloid cell populations.

Acknowledgements

This work is sponsored by the DoD Breast Cancer Research Program DAMD17-10-1-0318. We would like to thank Pfizer for supplying the CpG (PF3512767). We would like to thank Marv Ruona in Media Support Services for his expertise in the design and preparation of this poster.